Inhalation Toxicology, 17:189–197, 2005

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370590912743

Taylor&Francis
healthsciences

Effects of Subchronic Exposures to Concentrated Ambient Particles (CAPs) in Mice: II. The Design of a CAPs Exposure System for Biometric Telemetry Monitoring

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We modified, assembled, tested, and validated the versatile aerosol concentration enrichment system (VACES) developed by Sioutas et al. (1999) for use in a subchronic experiment that involved exposures of mice in vivo and of respiratory epithelial cells in vitro to concentrated ambient particles (CAPs). Since the labor-intensive nose-only exposure regimen is not an option in a long-term experiment, a whole-body exposure mouse chamber was designed specifically for use with the VACES. The exposure system consists of a stainless-steel (SS) tub with 32 cubicles (1 mouse per cubicle) separated by perforated SS sheets. The tops of these cubicles are covered with perforated plastic sheets to allow telemetry monitoring during the exposure. In each exposure chamber, perforated aluminum tubes are used to distribute CAPs evenly (within 2% difference) throughout the exposure chamber. The exhaust consists of perforated aluminum tubes covered with a urine shield. The modification to the original design of the VACES facilitated the operation of the system in a subchronic study. Mass flow controllers maintain a constant flow rate into the exposure chambers. For a sham control exposure, the identical system is used, except that a HEPA filter at the inlet to the VACES removes 98% of ambient particles. The entire system allows for simultaneous exposure of 64 mice to CAPs, with an equal number of sham-exposed mice as controls. Telemetry receivers have been modified so that 16 mice per group with electrocardiograph (EKG) transmitters can be monitored during exposure. Furthermore, a BioSampler is used to collect CAPs (one sample per day) for the in vitro exposures. In this article, the assessments of flow and particle distribution of the exposure chamber as well as the performance of the system during the subchronic exposure experiment are described.

The Harvard Six Cities Study (Dockery et al., 1993) and American Cancer Society (ACS) Cohort Study (Pope et al., 1995, 2002, 2004) have shown in cities with higher average $PM_{2.5}$ concentrations significantly increased annual mortality that was not explained by personal risk factors. The association between chronic exposure to $PM_{2.5}$ and increased mortality was also significant when the ACS and Six Cities studies were extended for an additional 9 yr (Pope et al., 2002, 2004; Laden et al., 2000). The PM effect estimates reported in these studies are much larger than the cumulative effects reported for acute PM exposure and mortality (U.S. EPA, 2004). This finding indicates that people who live in areas with elevated PM experience cumulative adverse health effects in addition to acute transient effects. Increased mortality is not the only adverse health effect associated with PM exposure; several cross-sectional studies of children (Dockery et al., 1989, 1996; Raizenne et al., 1996) have shown that children who live in cities with higher average $PM_{2.5}$ concentrations have more respiratory symptoms and decreased pulmonary function. Moreover, the Children's Health Study in Southern California has shown that chronic exposure to increased PM pollution is also associated with slower cumulative lung growth (Gauderman et al., 2000, 2002). Animal inhalation exposures to ambient aerosol do not generally yield measurable

Received 6 July 2004; accepted 5 August 2004.

This research was supported by the NYU-EPA Particulate Matter Health Effects Research Center (R827351) and by an NIEHS Center Grant (ES00260).

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adverse effects, due to the low PM concentrations. However, recent development of particle concentrators (Sioutas & Koutrakis, 1996; Sioutas et al., 1999; Kim et al., 2001a, 2000b; Kidwell & Ondov, 2001) allowed conducting exposures to ambient PM at much higher concentrations. Recent acute CAPs exposure studies (Saldiva et al., 2002; Cheng et al., 2003; Clarke et al., 2000; Gordon et al., 1998, 2000) provided some insights on the acute health effects. However, there are no reported studies on chronic CAPs exposure that would explain the outcome of the human epidemiological studies.

Based on these findings, the New York University (NYU) PM Center conducted the first subchronic animal inhalation studies extending over many months using CAPs in order to provide supplementary and complementary data analogous to that developed in the human cohort studies in cities with varying levels of fine PM. We completed two studies in March–September 2003 and February–May 2004, with daily 6-h exposures to CAPs for 5 days/wk. The main focus of these subchronic inhalation studies was on the direct and indirect cardiopulmonary effects of PM. This article focuses on the design and evaluation of the concentrator system used in these studies.

EXPERIMENTAL METHODS

Design of the Exposure System

The concentrator concept selected for these studies was the versatile aerosol concentration enrichment system (VACES) that uses the principle of the condensational growth of the ambient particles followed by virtual impaction to concentrate the aerosol (Sioutas et al., 1999). A complete evaluation of the VACES was presented by Kim et al. (2001a, 2001b). We have made several modifications to the VACES to improve its performance for our application and to facilitate its use for daily operation. The schematic of the entire system is shown in Figure 1.

Ambient aerosol is drawn at a flow rate of 330 L min^{-1} through a cyclone inlet (Aerotec 2) that removes most of the particles larger than 2.5 μm in aerodynamic diameter. The cyclone outflow is passed over the warm bath of water. The saturated aerosol then enters the condenser, where it is rapidly cooled to about 20°C, resulting in supersaturation. The particles growth is by condensation of water vapor onto the particle surface. We replaced the salt–ice slurry used in the condensation process of the original VACES with a chiller recirculating antifreeze (dual pump model 505-007, single pump model 9705, Niles, IL), which maintained the temperature at approximately −7°C. A dewpoint hygrometer (Hygro M1, General Eastern, Wilmington, MA) was used to monitor the water vapor pressure in the water bath, and temperatures of water-bath vapor and the chiller were recorded. Upon the exit of the condenser, the aerosol flow was split three ways, and the grown particles were concentrated using a virtual impactor operating at a major flow of 95 L min^{-1} and a minor flow of 5 L min^{-1}. The CAPs of the minor flow were diluted with filtered clean air (room air passed through silica gel, carbon canisters, and a HEPA filter to remove moisture, gaseous pollutants, and particulates) to 10 L min^{-1}, passed though the diffusion dryers (model 3062, TSI, Inc., St. Paul, MN), and then to the exposure chambers. Teflon filters (37 mm, 0.2 μm pore, Gelman Sciences, Ann Arbor, MI) used for gravimetric and

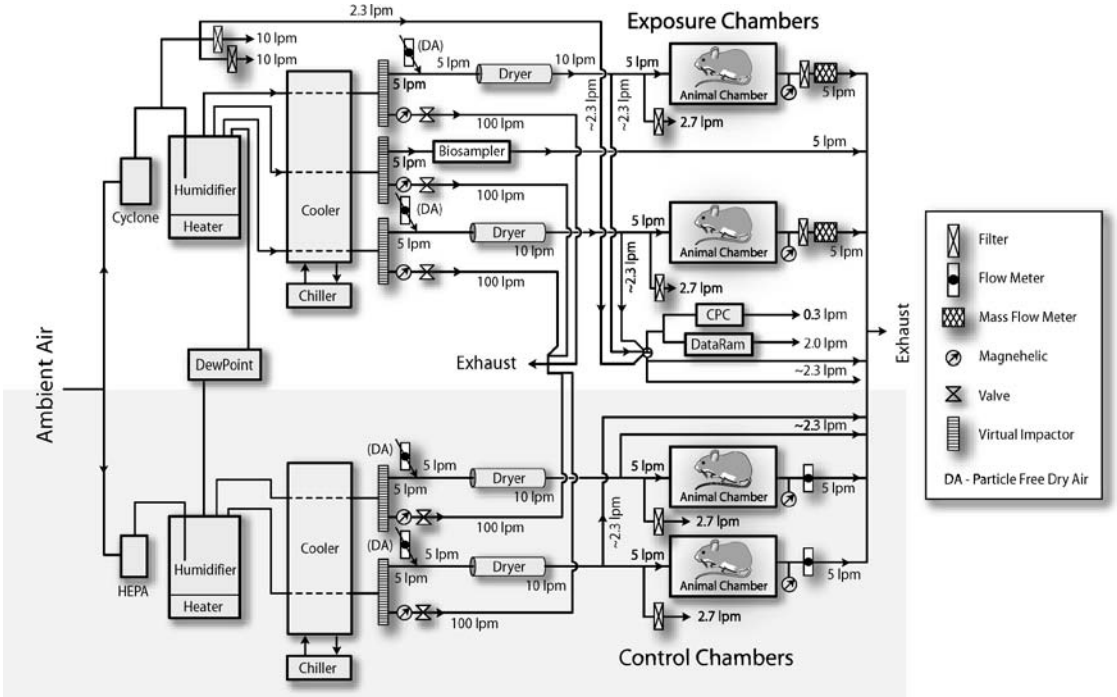

FIG. 1. The schematic diagram of the VACES exposure system.

elemental analyses were placed downstream of the cyclone inlet to collect ambient particulates, and downstream of the diffusion dryer to collect CAPs. Mass flow controllers (GFC 171, Aalborg, Orangeberg, NY) were used to maintain constant flow rates through the exposure chambers. For the sham control experiment, an identical system was used, except that a HEPA filter at the inlet to the VACES removed ambient particles.

Nose-only exposure of mice is a labor-intensive regimen, and is not an option in a long-term experiment. Instead, we adapted a whole-body exposure mouse chamber first used by Oldham et al. (2004) for use specifically with the modified VACES. The whole-body exposure unit (chamber) consists of four 5-gal (51 × 30.4 × 15.2 cm) stainless-steel (SS) tubs, each with 32 cubicles (1 mouse/cubicle) separated by perforated SS sheets (3 mm hole diameter, 40% open, staggered; McMaster-Carr, New Brunswick, NJ) constructed in our laboratory (Figure 2). In each exposure tub, 6 aluminum tubes, each 22 cm in length with 15 0.25-mm holes 13.5 mm apart, are bolted to the Plexiglas top to distribute CAPs flow evenly throughout the exposure chamber. At the bottom of the chamber, the exposure atmosphere is exhausted through 2 aluminum tubes, each 40 cm in length with 28 0.5-mm holes, which are covered with semicircular urine shields. The tops of the cubicles are covered with perforated polypropylene plastic sheets (3 mm hole diameter, 40% open, staggered; McMaster-Carr, New Brunswick, NJ) to prevent the animals from crawling over to the adjacent cubicles while allowing telemetry monitoring of 8 mice/chamber during the exposure. These plastic sheets also allow even distribution of the exposure atmosphere to cubicles. The entire system allows for simultaneous exposure of 64 mice to CAPs, with an equal number of sham-exposed mice as controls.

In our exposures, various strands of mice were implanted with heart-rate monitor transmitters (model TA 10, ETA F20, Data Sciences, St. Paul, MN). To monitor 8 mice/chamber during the exposure, each of the original Data Science receivers was modified by placing the antenna and electronic circuit boards into an 8.9 × 20.3 cm plastic enclosure box. Aluminum sheets were used to cover the inside walls of the enclosure box except a 5 × 5 cm opening. This opening allows the antenna to receive signals from the transmitter. Animals with transmitters were placed in every other cubicle along the perimeter of the exposure chamber. This arrangement, along with the perforated metal walls of the exposure cubicles and the aluminum sheets, minimizes cross-interference between adjacent animals with transmitters.

To assess the daily biological response due to changes in ambient PM, a BioSampler (SKC, Inc., Fullerton, CA) was used to collect CAPs for the in vitro exposure (Willeke et al., 1998; Kim et al., 2001a, b). The BioSampler was placed directly at the end of the virtual impactor. Since the particles emerging from the virtual impactor had grown to at least 2.5 μm in diameter, these particles were collected by impaction to the wall of the sampler without addition of other fluid as recommended by the manufacturer. The resulting particle suspension was then freeze-dried and later reconstituted to 500 μg/ml for in vitro exposure. Detailed cell culture, in vitro particle exposure, and subsequent assay for NF-κB activities are described elsewhere in this issue (Maciejczyk et al., 2005).

Evaluation of the Cyclone Inlet and Animal Chamber

The cyclone collection efficiency was validated using monodisperse fluorescent test particles according to the method described by Gordon et al. (1998). Briefly, the test particles (1.2–10 μm were generated from a stock solution of 2 ml oleic acid, 95 ml analytical-grade 100% ethanol, and 0.1 g fluorescein using a vibrating orifice generator (VOG, model 3450, TSI, Inc.). The outputs of the VOG were diluted with HEPA-filtered air

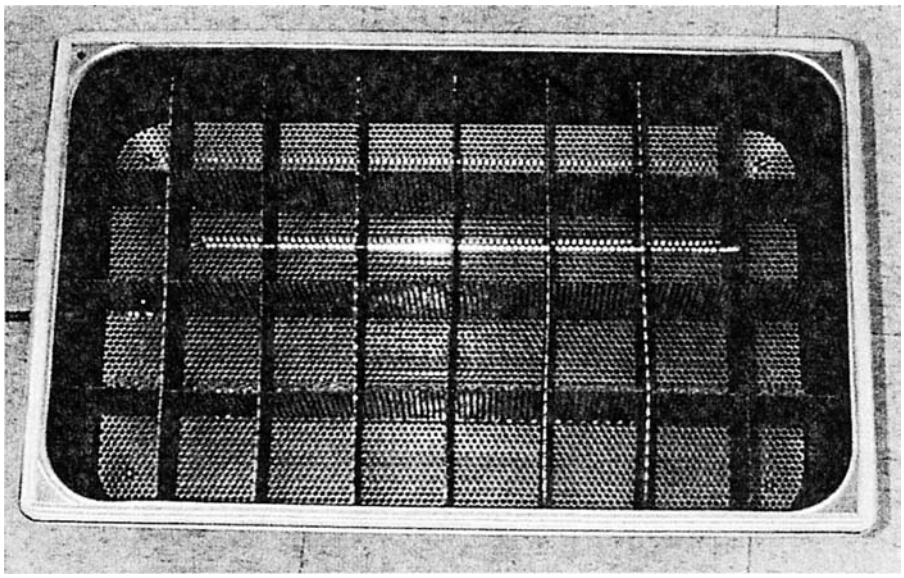

FIG. 2. Animal exposure chamber.

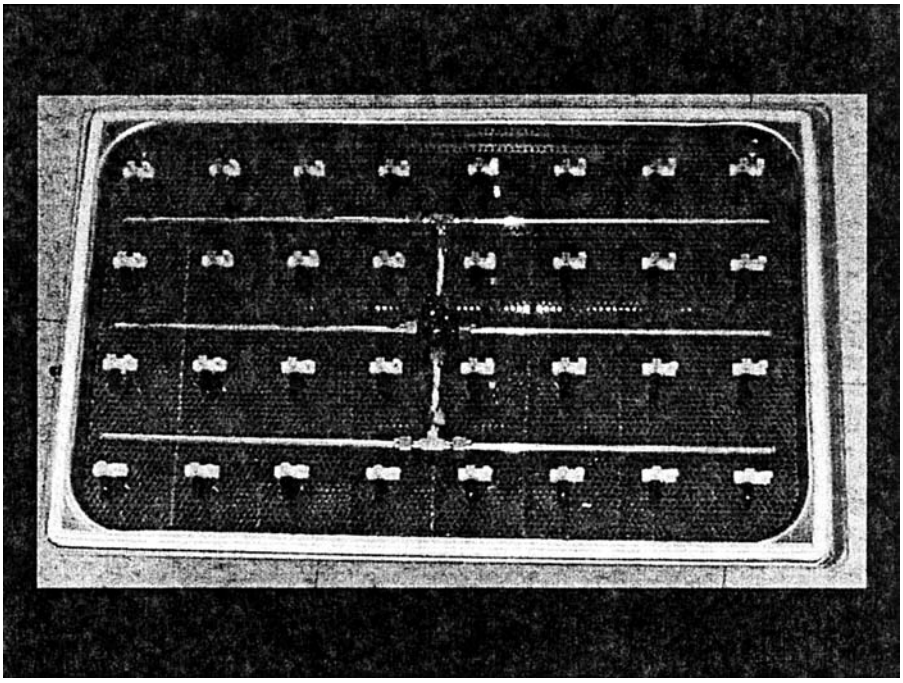

FIG. 3. Thirty-two three-way stopcocks with probes lid to test the particle distribution within the animal chamber.

prior to introduction into the inlet of the cyclone. Simultaneous filter samples were taken to collect particles entering and exiting the cyclone and the virtual impactors. Filters were sonicated in 0.1% NH_4OH extracting solution and the total fluorescence was measured using excitation and emission wavelengths of 480 and 514 nm, respectively (TD-700 fluorometer, Turner Designs, Sunnyvale, CA).

To ensure that the CAPs are distributed evenly inside the exposure chamber, 32 three-way stopcocks with probes to samples from the breathing zone of the animals in the cubicle were installed on the Plexiglas lid as shown in Figure 3. The 0.4–0.5 μm volume median diameter NaCl test particles were generated with a Collison nebulizer, and the concentration of particles in each cubicle was measured consecutively for 5 min with a DataRAM nephelometer.

Monitoring of the Exposure Atmosphere and Ambient Aerosol

For the subchronic exposure studies, the VACES exposure system was installed at our Sterling Forest Laboratory with an inlet on the second floor of the building facing south and southwest. The site selection criteria are discussed in a companion article by Lippmann et al. (2005). Outdoor air particles were drawn through a 2-inch-diameter SS inlet tube, with insect and rain trap, extended approximately 6 ft beyond the wall of the building. The composition of air and PM was measured at three locations: outdoor (referred as Outdoor), after the cyclone inlet (Ambient), and after the diffusion dryers (CAPs). Measurements included continuous PM mass concentration (Ambient and CAPs) with DataRAM nephelometer (MIE, Inc., Bedford, MA), daily integrated $PM_{2.5}$ mass in Outdoor with TEOM with automatic cartridge collection unit (ACCU) sampler (R&P, inlet temperature 50°C, inlet design was $PM_{2.5}$ sharp-cut cyclone), and Ambient and CAPs on Teflon filters, Ambient continuous black carbon (BC, Aethelometer, Andersen Instruments, Magee Scientific, AE-14 dual channel, wavelength for the BC channel 880 nm, absorption cross-section for channel 1 (BC) was 12.6, channel 2 (UV carbon) was 30), daily integrated black carbon (reflectometer, Andersen Instruments), Outdoor semicontinuous elemental (EC) and organic carbon (OC) (model 5400, Rupprecht & Patashnick, East Greenbush, NY), Ambient and CAPs continuous particle number concentration (CPC, TSI model 3020, St. Paul, MN), Outdoor O_3 (model 103-PC, Thermo Environmental Instrument, Inc.), Outdoor nitrogen oxides (model 8840, Monitor Labs.), and Outdoor SO_2 (model 8850, Monitor Labs.). Particle size distributions were measured using an SMPS system (electrostatic classifier model 307100, CPC model 3010, SMPS software 2.3, CPC software 1.0, CP count software 1.02, TSI, Inc., St. Paul, MN). Since only one SMPS system was available, the size distribution spectra were collected continuously but not concurrently after the cyclone inlet and the diffusion dryers. Average sampling period for each spectrum was 10 min. Temperature and humidity of Outdoor and CAPs atmospheres, as well as dewpoint at the virtual impactor outlet, were recorded every 15 min. All PM samples for gravimetric and elemental (via energy-dispersive x-ray fluorescence, ED-XRF) analyses were collected on Teflon filters. Filter samples were stored at constant temperature and humidity (21 ± 0.5°C, 40 ± 5% relative humidity) until analyzed. Filter mass was measured on a microbalance (model MT5, Mettler-Toledo, Inc., Highstown, NJ). Analysis for

34 elements followed by nondestructive XRF (model EX-6600-AF, Jordan Valley), and spectral software XRF2000v3.1 (U.S. EPA and ManTech Environmental Technology, Inc.). Then filters were analyzed by ion chromatography with a conductivity detector to determine the concentrations of water-soluble sulfate, nitrate, chloride, and phosphate (Dionex).

RESULTS AND DISCUSSIONS

Evaluation of the VACES Add-Ons

Using monodisperse fluorescein-tagged oleic acid particles, the Aerotec 2 cyclone operating at 330 L min^{-1} has a 50% cutoff size of 2 μm. Figure 4 presents the collection efficiency of the cyclone as a function of particle aerodynamic diameter. The particle cutoff curve of the cyclone is not sharp and approximately 15% of the particles >10 μm in diameter remain in the exposure atmosphere. The geometric standard deviation (σ_g), a measure of the sharpness of the collection efficiency curve, was calculated as a ratio of aerodynamic diameters of the fitted curve at 84% and 50%. The value of σ_g for this cyclone is 3.8, which is not a sharp separation quality. However, given that the size distribution of the ambient air is typically in the accumulation mode and that the majority of the coarse mode particles (between 2.5 and 10 μm) were removed by the cyclone, the small penetration of these coarse particles would not be expected to have a large impact in this study.

We compared the mass concentrations collected after the cyclone inlet (ambient mass concentrations) to those measured by the TEOM equipped with ACCU sampler (Outdoor mass concentrations) for 2003 study. As shown in Figure 5, there is a very good agreement between these measurements: The slope of Ambient versus TEOM is 1.017 and R^2 is .910, and the slope of Ambient versus ACCU filter is 0.962 with R^2 is .757. This agreement indicates that the concentration of particles larger than 2.5 μm, which contribute to Ambient concentration, is low at our Sterling Forest site.

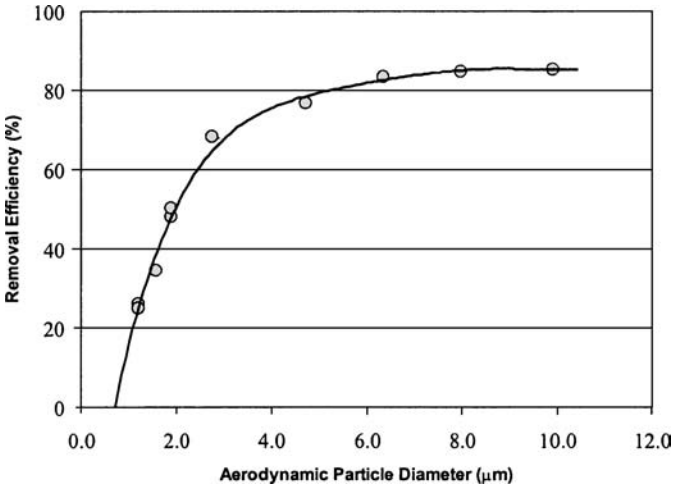

FIG. 4. Removal efficiency of Aerotec 2 cyclone inlet as a function of aerodynamic particle diameter. The line is a regression fit.

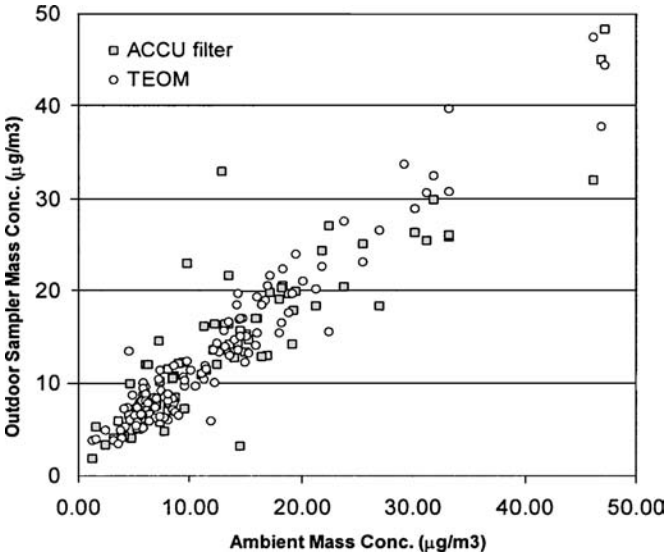

FIG. 5. Comparison of mass concentrations ($\mu g/m^3$) collected after the cyclone inlet (Ambient mass concentration) to ones collected by the TEOM equipped with an ACCU filter (Outdoor mass concentration).

The result of the test of particle distribution within the animal chamber is shown in Figure 6. In each cubicle, the NaCl concentration was measured sequentially, starting from upper left, and then was normalized to the upper left cubicle. Figure 6 shows the schematic diagram of the animal chamber as in Figures 2 and 3, with the ratio of the particle concentration for each cubicle. The results of the NaCl test indicated that particle concentrations were distributed uniformly in the chamber with a mean of 0.98 and a standard deviation of 0.06.

Particle Size Distribution

Particle size distribution is an important characteristic of ambient and exposure aerosol. Kim et al. (2001a, 2001b) discussed in detail the preservation of ambient particles by size and

1.00	0.97	0.89	0.96	0.92	0.90	0.94	0.91
0.99	0.96	1.00	0.98	0.94	0.95	0.96	1.04
1.10	1.16	1.02	0.96	1.05	1.02	1.06	1.04
1.14	1.03	1.08	0.93	0.91	0.94	0.97	0.93

FIG. 6. Ratio of particle distribution within the schematic diagram of animal chamber shown in Figures 2 and 3, normalized to the upper left cubicle.

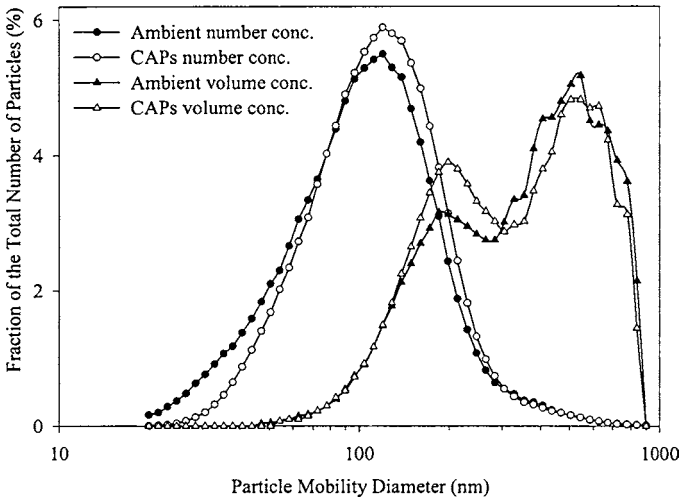

FIG. 7. Number and volume size distributions of particles before (Ambient) and after (CAPs) the VACES measured by SMPS. Each spectrum is an average of 8 spectra collected over a 2-h period.

fractal dimensions by VACES utilizing a micro-orifice impactor and SMPS system. We continuously measured the number and volume size distributions of ambient aerosol upstream of the VACES immediately after the cyclone inlet and of the concentrated aerosol downstream of the diffusion dryers using SMPS during the daily exposure. The ambient and CAPs spectra were normalized to the total particle number and averaged. Figure 7 shows an example of the averaged particle fraction of total concentrations recorded at each SMPS particle mobility diameter. The results indicate that both number and volume size distributions are reasonably well preserved during the concentration enrichment process. It appears that either VACES concentrates larger particles more efficiently, or there are moderate losses of ultrafine particles <40 nm. The average number mean diameters were 123 $\pm$ 12 nm for Ambient and 135 $\pm$ 2 nm for CAPs aerosol. Although the volume size distribution is bimodal, the mean diameter was calculated as 405 $\pm$ 23 nm for Ambient and 389 $\pm$ 2 nm for CAPs. From similar measurements during different exposure days, we established that the number mean diameter increases on average 4 $\pm$ 6% when passed through VACES, while volume mean diameter decreases on average 5 $\pm$ 3%. Considering that these are whole-day averages of the nonconcurrently collected spectra, these differences in size distributions are quite acceptable for animal exposures.

Concentration Enrichment Factor

In each of the sampling line of the VACES, particles were concentrated from the flow of 100 L min^{-1} to a minor flow of 5 L min^{-1}, and then diluted to 10 L min^{-1}. Thus the ideal concentration enrichment factor (CEF) is 10. During the exposures, the CEF, defined as the ratio of the concentrations measured after the diffusion dryer to these after the inlet cyclone (CAPs/Ambient), was monitored by DataRAM and CPC. We concluded that the optimal operational CEF is 8–10 for both mass and particle number concentrations. When the system was manipulated to increase mass CEF, the particle number CEF decreased due to the wall losses of excessively grown particles; conversely, particle number CEF could be increased at the expense of decreased mass CEF. Table 1 shows the concentration enrichments measured by DataRAM, CPC, and gravimetric and elemental analysis, as well as a summary of measurements of outdoor temperature and humidity. Two completed exposure studies were conducted at different seasons, giving us the opportunity to evaluate system performance at different meteorological conditions. During the 2003 study, average outdoor temperature was 18.3°C with percent relative humidity (%RH)

TABLE 1
Summary of mean daily parameters measured during 2003 and 2004 studies

	Mean	Median	Standard deviation
	2003		
Exposure CEF			
DataRAM	9.0	10.3	6.3
Gravimetric mass	8.9	8.7	2.2
Mean of 14 elements[a]	9.1	9.0	1.9
Saturation ratio	1.79	1.75	0.20
Sham CEF DataRAM	0.20	0.16	0.16
Outdoor			
Temperature (°C)	18.3	19.8	6.2
%RH	68.3	71.0	20.2
Exposure chamber %RH	43.9	45.4	11.5
O_3 (ppm)			
Ambient	32	29	15
Exposure chamber[b]	10	7	10
NO_2 (ppb)			
Ambient	8.1	7.2	5.6
Exposure chamber[b]	4.4	3.7	3.1
	2004		
Exposure CEF			
DataRAM	8.9	9.1	3.4
CPC	6.7	7.2	3.6
Saturation ratio	1.77	1.76	0.11
Sham CEF			
DataRAM	0.23	0.15	0.32
CPC	0.08	0.02	0.36
Outdoor			
Temperature (°C)	5.5	5.3	6.1
%RH	59.4	57.7	21.3
Exposure chamber %RH	35.1	37.3	7.5

[a]Only values above the detection limit (3σ) were included in the mean.

[b]Concentrations as measured in 2:1 diluted exposure atmosphere.

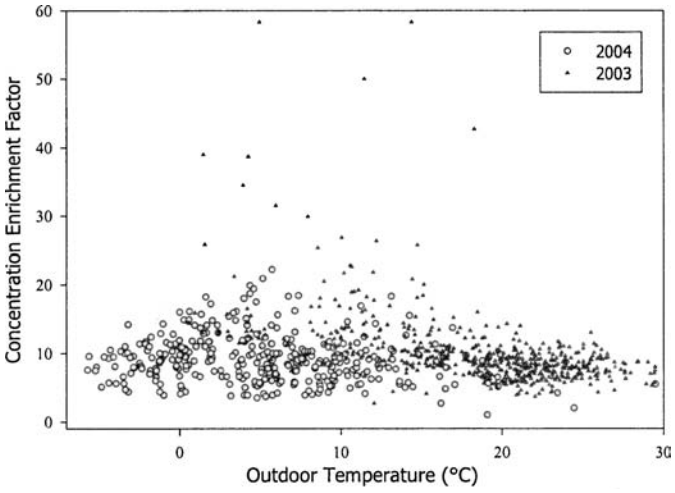

FIG. 8. Hourly concentration enrichment factor from RAM measurements in 2003 and 2004 exposures.

of 68.3. The 2004 study was conducted in a winter–spring season of colder and drier outdoor conditions (Table 1).

In the 2003 study, it was noted that at lower outside temperatures, the hourly mass CEF was less stable, and, as shown in Figure 8, lots of scattering of data was observed. This was caused by cold incoming air cooling the water bath, resulting in less efficient saturation. The 2003 hourly CEF data was split into 5°C intervals, a linear regression was performed and the variation of the residuals was calculated for each temperature interval. The results (shown in Table 2) indicated that less variation is observed when the outdoor temperature increases to 15–20°C. Thus, we installed a preheater, which was preset to 18°C, on the incoming aerosol pipe upstream of the cyclone. The preheater improves the efficiency of the system by allowing the air stream to saturate more quickly inside the water bath.

TABLE 2
Variation of the hourly concentration enrichment factor residuals in specified temperature range during 2003 and 2004 studies

Temperature interval (°C)	Standard deviation residuals	Number of measurements
	2003	
0–5	11.29	25
5–10	5.52	35
10–15	10.15	99
15–20	3.72	137
20–25	1.84	212
25 and up	1.49	71
Total 0–18	8.17	230
Total 18 and up	2.63	349
	2004	
All data	3.39	348

The 2003 variation of residuals of hourly CEFs in temperature range up to 18°C was 8.17, while above 18°C it was 2.63. With a preheater, the variation in CEF in 2004 study (conducted during the colder months period) was quite low at 3.39 and less scatter was observed.

The results of daily CEFs calculated based on the gravimetric and elemental analysis of the filter samples are shown in Table 1. Comprehensive discussion of elemental analysis is presented in a companion article (Maciejczyk et al., 2005) in this issue. Although the XRF analysis was done for 34 elements, elemental CEFs were calculated only for the detected elements. In our treatment of XRF data, the element was considered to be detected if the value was larger than three times the uncertainty of the measurement. Overall, only 14 elements were detectable. Table 1 shows the mean, median, and standard deviation of the arithmetic averages of each detected element. All of the parameters CEFs but for the particle number are in the optimal 8–10 range. Additionally, there is a very good agreement between CEFs for the DataRAM and gravimetric mass in 2003 study: the mean CEF was 9.0 and 8.9, respectively. The standard deviation of the DataRAM CEF is higher than gravimetric mass CEF because DataRAM continuously recorded high and low concentrations are averaged by the long 6-h collection on the filter. As shown in Figure 9, the mass concentration range of filter measurements is lower that of DataRAM. However, there are high correlations of mass concentrations in Ambient and CAPs samples measured with DataRAM and filters: R^2 is .932 and .934, respectively.

For the control animal chambers, the HEPA filters were used to remove the ambient PM from the ambient air. As with the exposure animal chamber, the particle count and mass concentration were monitored continuously, and the CEFs were calculated. Here, the CEFs are the indicator of efficiency of particle removal and, clearly, are expected to be well below 1. It should

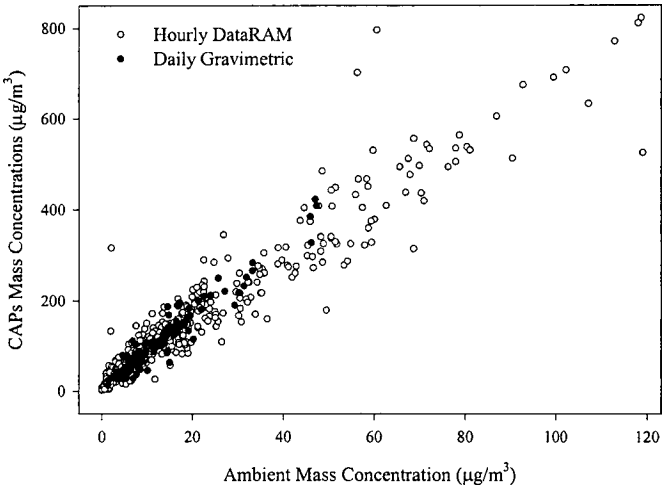

FIG. 9. Ambient and CAPs mass concentrations measured by the hourly averaged DataRAM and gravimetric analysis of daily filter samples.

be noted that at very low concentrations DataRAM measurements approach the detection limit of this instrument, and are less reliable. The results, shown in Table 1, indicate that the HEPA filter eliminated 98% of particles in the sham exposure atmosphere.

The VACES is designed to concentrate particles but not gaseous pollutants, which theoretically should remain unchanged. However, the gases could be scrubbed out by the water vapors during the humidification process. We measured the O_3 and NO_2 concentrations before the water bath and in the exposure chambers. These measurements were conducted daily in July 2003, and involved 10 min of continuous monitoring every other hour. The mean, median, and standard deviation of daily averages are shown in Table 1. Daily ambient mean concentrations of ozone reduced from 32 ppb to 10 ppb when passed through the VACES. The respective reduction of mean concentrations of NO_2 was from 8.1 ppb to 4.4 ppb. However, the NO_2 concentrations were very close to the detection limit of 8 ppb of the analyzer, thus making further comparison limited to 1 day only when the Ambient NO_2 was 23.5 ppb and CAPs 10.8 ppb. Taking into the account the 2:1 dilution of the CAPs air stream, these results indicate that on average $22 \pm 100\%$ of O_3 and 8% of NO_2 are lost in VACES. We concluded that the loss of gaseous pollutants is minimal during the particle enrichment process.

Saturation Ratio

Another system performance index is the saturation ratio (SR), which is a ratio of the actual vapor pressure to the saturation vapor pressure at a given temperature. For our system it was practical to calculate the daily SR as a ratio of water vapor pressure at the temperature above the water bath to water vapor pressure at the temperature after the cooler (20°C). The means of average daily SRs were 1.79 ± 0.20 and 1.77 ± 0.11 during the 2003 and 2004 exposures, respectively. The variation in SRs was reduced in 2004 as the result of the installed preheater. Vapor loss due to condensation on the chiller walls and onto particles will reduce that ratio; thus, values calculated here are maximum theoretical SRs. It could be shown that a saturation ratio of only 1.25 is needed to grow particles as small as 0.01 μm by condensational growth (Hinds, 1982).

The outflow of concentrator designated for animal exposures is passed through the diffusion dryer that reduces the relative humidity of the aerosol from supersaturation to less than 50% in order to dry the particles to their original size. As shown in Table 2, the mean daily average relative humidities measured in the animal exposure chambers were 43.5 ± 11.5 and 35.1 ± 7.5 in 2003 and 2004, respectively.

CONCLUSIONS

This article focuses on the evaluation of two modified VACES (exposure and sham atmospheres) used in the in vivo and vitro studies. Each of the modified VACES can be used to expose up to 64 mice to CAP simultaneously. The ease of use allows the modified VACES to be used in a long-term study. EKG or blood pressure can be monitored simultaneously during the exposure for up to 16 animals through telemetry. The distribution of particles in a 32-cubicle chamber is uniform. Sham control was conducted in parallel to the CAP exposure using an identical system with PM removed by a HEPA filter upstream of the humidifier, which effectively reduces 98% of particle concentrations. CAP can be collected using a BioSampler for additional in vitro exposures or instillation studies.

There was a good correlation of mass concentrations in samples collected after the nonsharp VACES inlet cyclone and outdoor measurements with TEOM and ACCU sampler equipped with sharp-cut $PM_{2.5}$ inlet. The concentration enrichment factors based on continuous mass and particle numbers, as well as integrated mass and individual elements measurements, were very close to the theoretical value of 10. The performance of the system was improved by installing the preheater for the incoming air flow. The losses of the gaseous pollutants were small during the enrichment process.

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